April 20, 2005

US Patent and Trademark Office
US Department of Commerce
Appn. Number: 10/007,489
Appn. Filed: 12/05/2001
Applicant: Elizabeth Gay Frayne

Title: "Microbial Production of Phosphorothioate Substituted DNA, RNA, and Oligo Mixtures"

Examiner: Devesh Khare, PhD, JD
Art Unit:1623

RE: Request for Continued Examination.

Dear Sir,

Claims 1-5 have been rejected. The reason for rejection has been stated in the advisory action dated 4/14/2005 that steps 1 and 3 of claim 1 require further search and/or consideration. The claims were originally rewritten to create more positive steps in describing how to carry out the invention. Note that in office action 10/18/2004 it was conceded that the prior art revealed no references that could appropriately be applied to claims 1-5, claiming a method for the in vivo incorporation of thiophosphate into precursor nucleotide pools of ds DNA, ss DNA, and/or RNA and ultimately into polymer form resulting in modified internucleotide linkages. Thus the novelty of the invention is apparent. The utility of the parent application is in the facile production of bulk quantities of nuclease resistant DNA and/or RNA, i.e. plasmids, phage, etc. Such DNAase resistant plasmids may be useful for stabilizing DNA vaccines or gene therapy plasmids used to treat humans. Further processing of DNase resistant ss phage DNA in vitro can lead to the production of mixtures of antisense DNA oligos for use in gene ablation studies and potential pharmaceutical treatments.

The essence of the patent application and related divisional applications relies on a very novel method of chemically modifying nucleic acids in vivo by culturing cells or organisms in media that has the normal phosphate used for making nucleic acids substituted with thio-phophosphate. The novelty of the process is in the demonstration that thiophosphate is readily taken up by cells and incorporated into nucleotide precursor pools that feed into the synthesis of nucleic acids. It is known from in vitro studies (Eckstein, 1985) that nucleotide triphosphates modified with an

alpha thio-phosphate can be utilized by bacterial DNA and RNA polymerases in vitro. My work demonstrates that thio-phosphate can be incorporated into nucleotide precursor pools and eventually into nucleic acids. While I don't directly measure the presence of modified nucleotides in nucleotide pools my work does show that the addition of thiophosphate to culture media leads to the synthesis of nucleic acids that are resistant to degradative nucleases in vivo and in vitro upon isolation. This is what would be expected from the successful incorporation of thio-phosphate into nucleic acids resulting in phosphorothioate substituted nucleic acids.

The present application focuses on the production of nucleic acids that are highly substituted with thiophopshate. This requires using culture media largely depleted of normal phosphate and replaced with thio-phosphate. The utility is in producing recombinant DNA molecules that are highly resistant to DNase in an easy and economical manner. To do this recombinant DNA molecules are replicated in bacterial cells grown in thio-phosphate containing media. This results in DNA plasmids or phage that can be isolated from such cultures that are highly substituted with phosphorothioate. The versatility of this method is apparent in that any plasmid or phage in any bacteria can adapted to this approach. I have further shown that the utility of the method is not restricted to bacteria but can be applied to complex eukaryotic systems as well. Gold fish incubated in thio-phosphate containing water incorporate thio-phosphate into genomic DNA.

Another utility of the present application is in the isolation of nucleic acids as intact species for analytical purposes. The present method facilitates the isolation of nucleic acids by protecting the nucleic acids from degradative conditions during isolation. This may be particularly useful for isolating mRNA from microbial cultures which is otherwise difficult to do. The second divisional is directed more at the utility of stabilizing RNA to increase protein synthesis in cells and enhance mRNA isolation.

The method of using micro-organisms to chemically modify nucleic acids is clearly distinct from chemical synthetic methods (most of which are not stereoselective) and in vitro enzymatic methods which are cumbersome and expensive. In fact these methods are not used commercially to make preparations of modified DNAs larger than 100 nucleotides. In this regard some genes are created artificially in vitro but are then amplified in bacteria. The ability to chemically modify bacterial DNA during amplification is truly a significant advance in synthetic methods.

Please under MPEP 707.07(j) the pro se applicant requests that if the Examiner fines patentable subject matter disclosed in this application, but feels that

applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant

To assist in this processing this patent application I would like to further request a supervisory patent examiner review.

Please note as requested I have included a statement of the summary of the interview between the examiner and myself on 3/25/05. I am also including a statement of the interview had on 4/18/05.

Respectfully submitted,

Elizabeth Frayne
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STATEMENT OF THE SUBSTANCE OF THE INTERVIEW WITH THE EXAMINER DEVESH KHARE MARCH 25, 2005.

Inventor and applicant pro se: Elizabeth Gay Frayne
Application No. 10/007,489
Examiner: Devesh Khare
Date of Interview: 3/25/05
Type of interview: telephonic
Exhibits: none
Prior art discussed: none
Agreement with respect to claims: not applicable

I acknowledge and agree with the examiner as to the substance of the interview. The original application at the request of the examiner was divided into three separate applications. The divisional application 10/760,156 filed 1/20/2004 was intended to be kept non-published as indicated in the application data sheet. The request for non-publication was denied because there was no statutory signature requesting this. I wished to alert the examiner that the divisional was going to be published soon and to urge him to write the claims for me as he saw fit. I was unclear as to why it was taking so long as the novelty of the invention was not contested. He said he would need to get assistance from his supervisor to help me with the claims as an applicant pro se and would get back to me.

Elizabeth Gay Frayne

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Interview Summary

Application No.	Applicant(s)	
10/007,489	FRAYNE, ELIZABETH GAY	
Examiner	**Art Unit**	
Devesh Khare	1623	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Devesh Khare*. (3)_____.

(2) *Elizabeth Frayne*. (4)_____.

Date of Interview: *25 March 2005*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☐ No.
If Yes, brief description: _____.

Claim(s) discussed: _____.

Identification of prior art discussed: _____.

Agreement with respect to the claims f)☐ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *An advisory action has been issued.*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Examiner's signature, if required

STATEMENT OF THE SUBSTANCE OF THE INTERVIEW WITH THE EXAMINER DEVESH KHARE APRIL 18 th, 2005.

Inventor and applicant pro se: Elizabeth Gay Frayne
Application No. 10/007,489
Examiner: Devesh Khare
Date of Interview: 3/25/05
Type of interview: telephonic
Exhibits: none
Prior art discussed: no specific prior art
Agreement with respect to claims: not applicable

In response to the advisory action received 4/14/2005, I contacted the examiner to see if the matter could be easily resolved. Claim 1 steps 1 and 3 were discussed briefly and the examiner indicated that more time was needed to further search the prior art. I was not clear as to how the modified claims created new issues but the language had changed so as to create the description of the invention in a positive manner. The examiner advised that I file an RCE and I agreed to do this. I also indicated my willingness to have the examiner write the claims for me. He suggested that I send a description of the essence of the invention by email to help in this matter.

Elizabeth Frayne

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